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Original article

Juvenile hormone promotes flight activity in drones (*Apis mellifera carnica*)

S de Oliveira Tozetto, A Rachinsky, W Engels*

Zoologisches Institut, LS Entwicklungsphysiologie, Universität Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, Germany

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Summary — Drones of *Apis mellifera carnica* received a single topical application of juvenile hormone III (5 or 10 µg in acetone-hexane) a few days after emergence. This led to flight attempts 2 - 3 days earlier than in untreated control drones which were kept in the same colony. It is clear that juvenile hormone in drones acts as a flight stimulus, comparable to its function in worker bees where foraging activity depends on high juvenile hormone levels in the hemolymph.

***Apis mellifera carnica* / drone flight activity / juvenile hormone / flight stimulus / premature flight attempts**

INTRODUCTION

Juvenile hormone (JH) is known to be involved in many aspects of female insect reproduction (Engelmann, 1990), a fact reported by Wigglesworth (1936) in his pioneering studies on *Rhodnius prolixus*. In contrast to this large body of information, surprisingly little is known about the role of juvenile hormone in male reproduction (Wyatt and Davey, 1996), except that this hormone accelerates the maturation of the accessory glands in adults (Happ, 1992). During early stages of gonadal differentiation in late larval development, the rate of juvenile hormone production in drones of *Apis mellifera carnica* (Hartfelder et al, 1993) was found to resemble workers more than queens (Rachinsky et al, 1990). In pupal drones, no juvenile hormone synthesis could be detected (Tozetto et al, 1995). According to van Laere (1971), the corpora allata (CA) are smaller in adult drones than in queens and workers.

An increase in juvenile hormone III content of drones from 4 days after emergence was described by Rembold (1987). Recently we found a re-activation of the CA in young

* Correspondence and reprints
Tel: (49) 7071 2974650; fax: (49) 7071 296950; e-mail: wolf.engels@uni-tuebingen.de

adult drones (Tozetto et al, 1995). In the 9 days following emergence, a steady increase in juvenile hormone synthesis was observed, followed by a decrease. Presumably a corresponding increase in juvenile hormone hemolymph titer occurs. Therefore, we hypothesized that juvenile hormone must also have functions in the imaginal phase of male bees, perhaps in part similar to its manifold effects in adult workers (Robinson, 1992).

During the first week of their adult life, drones stay inside the nest and are fed on honey and jelly secretions by nurse bees (Szolderits and Crailsheim, 1993). During the second week, they become sexually mature (Mindt, 1962) and begin to fly out of the hive (Drescher, 1969), now feeding themselves on comb honey (Wachsmann and Crailsheim, 1994). Nuptial flights to drone congregation areas (Ruttner and Ruttner, 1965) are then continued till the end of life, approximately 30 days after emergence (Howell and Usinger, 1933; Fukuda and Ohtani, 1977).

This temporal switch from intranidal to extranidal activities in the course of a drone's sexual maturation resembles the house bee to field bee transition in imaginal workers (Ruttner, 1992). First flight activity occurs during a worker's third week after emergence and was shown to depend on elevated levels of juvenile hormone hemolymph titer (Robinson et al, 1989), caused by an increased rate of the hormone synthesis (Kaatz et al, 1992). By juvenile hormone application, premature flight (Jaycox, 1976; Rutz et al, 1976) and foraging activity (Robinson, 1985; Robinson and Ratnieks, 1987) can be elicited in worker honey bees.

We hypothesized that the hormone may likewise promote flight activity in young drones (Tozetto et al, 1995). This was tested by application a few days after adult eclosion. The data presented here show that treatment leads to early flight attempts of drones.

MATERIALS AND METHODS

All the experiments (table I) were run during the late swarming season in June until Mid-July of 1995, a period of warm and sunny weather with uninterrupted drone flight activity. Drone combs were collected from strong *Apis mellifera carnica* colonies in the experimental apiary of the University of Tübingen. Shortly before emergence of adults, the combs were placed in an incubator. Groups of drones emerging within an 8 h period were given a cohort specific mark. A total of about 40 drones of two cohorts of the same age was introduced per queenright Kirchhainer nucleus (fig 1); the Kirchhainer nucleus is a nucleus colony with four small frames and about 2 000 worker bees. The final experiments were run with 12 such nuclei. When 2 - 5 days old, all drones of a particular nucleus were removed. Fifteen or 20 drones of a cohort received a single dose of 10 μg juvenile hormone III (Sigma), dissolved in 2 μL of acetone-hexane (9:1), by topical application onto the abdomen without narcosis. In a few cases, only 5 μg of juvenile hormone were applied. Since treatment of adult drones with solvent only (acetone-hexane) is known to have no effect on hormone titer (Tozetto and Rachinsky, 1997), such controls were not applied here. After treatment, drones were kept for about 30 min in an incubator to evaporate the solvent before being reintroduced into their nucleus. Fifteen control drones of the second cohort in the same nucleus remained untreated (table I). Thus a total of 35 drones was kept in each nucleus colony after the treatments. With this low number of drones, losses were widely prevented. Nevertheless, only the 12 colonies without drone mortality were used for the experiments.

Flight activity of drones was observed daily, from late morning through early afternoon, over a period of 2 weeks after emergence. In earlier experiments, we observed that unpredictable numbers of drones got lost in free flying colonies (Tozetto et al, unpublished data). Therefore, in the present study only flight attempts were recorded. This was controlled by means of an entrance vestibulum placed at the front of the Kirchhainer nucleus (fig 1). The drones could enter the vestibulum but not return, because of an unidi-

Table I. Effects of juvenile hormone III treatment on the first flight activity in young drones. In each of the 12 nuclei colonies a total of mostly 20 treated and 15 control drones, all emerged the same day, were kept together. Within the series of different treatments, the 12 nuclei are listed according to age of the drones at the time of hormone application and of first flight attempts, respectively. The seven cohorts of control drones are listed in analogous order.

Treatment of drones	*Number of drones per nucleus colony*	*Age at JH application*	*First record in the vestibulum*	*Time in between*
		(days after emergence)		*(days)*
10 µg JH III	15	2	4	2
	15	2	4	2
	20	3	6	3
	6	3	7	4
		$\bar{x}$ = *2,5*	$\bar{x}$ = *5,3*	$\bar{x}$ = *2,8*
5 µg JH III	20	3	5	2
	20	3	6	3
		$\bar{x}$ = *3,0*	$\bar{x}$ = *5,5*	$\bar{x}$ = *2,5*
10 µg JH III	20	4	5	1
	20	4	5	1
	20	4	5	1
	20	4	6	2
	20	5	6	1
	13	5	6	1
		$\bar{x}$ = *4,3*	$\bar{x}$ = *5,5*	$\bar{x}$ = *1,1*
Controls*	30	-	6	
	30	-	7	
	20	-	8	
	20	-	8	
	20	-	9	
	20	-	9	
	50	-	9	
			$\bar{x}$ = *8,0*	

* The seven cohorts of control drones were splitted into groups of 15 which were added to each of the 12 nuclei containing JH-treated drones of identical age.

rectional drone exit. In addition, a queen excluder screen was placed over the entrance of the vestibulum so that the drones could not fly out. However, drone exit and vestibulum could be easily passed through by worker bees in either direction. Entering the vestibulum was considered a flight attempt. The drones had to stay there to be recorded only once. Usually we observed that all individuals attempting to fly out entered the vestibulum at more or less the same time. When after about 2 - 3 h flight attempts ceased, the drones in the vestibulum were collected and returned into their nucleus. The total number of treated and untreated drones found per day in the vestibulum of a particular nucleus was recorded.

Fig 1. Nucleus colony with vestibulum in front of it. Drones undertaking a flight attempt were confined in the vestibulum. Only workers could pass the entrance equipped with a queen excluder screen.

The difference in age at the first flight attempts was statistically evaluated by means of the χ^2 test.

RESULTS

Untreated drones did not appear in the vestibulum during the first 5 days after emergence (table I). On day 6, only one drone entered the vestibulum in each of 2 of the 12 colonies. On days 7 and 8, a few drones were found there. From day 9 after emergence, 20-30% of the drones were recorded daily in the vestibulum (fig 2). Attempted drone flight activity dropped sharply by the end of the second week.

Juvenile hormone-treated drones entered the vestibulum considerably earlier than controls. By day 4 after emergence, individual drones were recorded in the vestibulum of each of 2 of the 12 colonies. One day later, in four more colonies a few treated drones went into the vestibulum (table I). On days 6 and 7, in all colonies 12 - 15% of the treated drones were seen in the vestibulum (fig 2). From day 8 to day 12, the frequency of juvenile hormone-treated drones that showed flight activity ranged around 25% in all colonies. From day 13 on, the number of flight attempts decreased.

If drones received a single hormone application soon after emergence, the premature flight activity was recorded within 2 to 3 days. These flight attempts occurred significantly earlier than in the controls ($P <$ 0.02). However, if the hormone treatment was done at an adult age of 4 - 5 days, some drones entered the vestibulum the next day (table I). This was also significantly earlier than in the controls ($P < 0.01$).

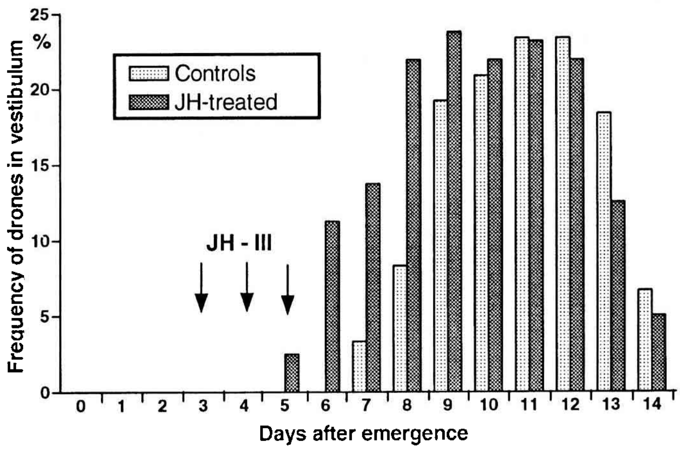

Fig 2. Temporal pattern of the daily flight attempts in juvenile hormone III-treated (n = 160) and untreated (n = 120) young drones, kept simultaneously in mixed groups of 35 individuals per nucleus colony in eight nuclei in total. The experimental drones received a single application of juvenile hormone III. The dosage was 5 µg in two nuclei and 10 µg in six nuclei. The age at application is indicated by arrows. Since no dosage and age effects were observed, all the data were accumulated in the same graph.

DISCUSSION

The recently detected reactivation of the CA in young drones (Tozetto et al, 1995) suggests a functional role for juvenile hormone in adult male honey bees. The data presented here support our assumed function of the hormone as a promotor of flight in drones, as previously shown in workers (Robinson, 1992). The steady increase in the hormone production by the CA of young drones until day 9 after emergence (Tozetto et al, 1995) is thought to result in a high juvenile hormone hemolymph titer. The hormone application presumably results in a precocious increase in titer. This physiological condition may stimulate sexual maturation in general (Mindt, 1962) and induce the behavioral switch from intranidal to extranidal activities in males, in particular the initiation of orientation loops and nuptial flights to drone congregation areas (Oertel, 1956; Drescher, 1969; Ruttner and Ruttner, 1965).

We hypothesize that drone flight activity depends on a critical juvenile hormone level in the hemolymph. By topical treatment the threshold titer may be attained earlier than normal, and premature flight attempts will be initiated. In fact, if juvenile hormone was applied on day 4 or 5 after emergence, the drones became active the next day. If, however, very young drones were treated, this elicited drone flight activity only 2 to 3 days later (table I). In all the treated drones, flight attempts occurred significantly earlier than in the controls, indicating a distinct effect of the hormone as a promoter of flight.

This effect is comparable to the role of juvenile hormone in adult worker bees. Their flight and foraging activities depend on a high hormone titer in the hemolymph attained about 15 - 20 days after emergence. Precocious outdoor activities have been likewise induced by juvenile hormone application in worker bees (Robinson and Ratnieks,

1987) and by methoprene treatment in drones (Giray and Robinson, 1996). Thus a common function of the hormone as a hormonal factor regulating extranidal behavior and in particular flight activity in social bees is hereby demonstrated. Earlier experiments on the juvenile hormone effects on mating flights of young honey bee queens, however, gave no clear results (Regel, 1978) and should be repeated.

Additional functions of the juvenile hormone in young drones may be the control of the final steps in spermatogenesis (Kerr and Silveira, 1974) and of mucus production by the accessory glands (Koeniger and Koeniger, 1991). Both are known to occur within a period of about 10 days after emergence and are essential for the attainment of full sexual maturity of a male bee.

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Résumé — L'hormone juvénile stimule l'activité de vol des mâles (*Apis mellifera carnica*). Afin de vérifier l'hypothèse selon laquelle l'hormone juvénile (JH) stimule l'activité de vol chez les abeilles en général, des mâles âgés de 2 à 5 jours ont reçu une application de 5 ou de 10 µg de JH III. Les mâles ont été marqués à l'émergence et introduits dans des ruchettes de Kirchhain au sein de petites colonies (2 000 ouvrières et une reine). L'activité de vol des mâles a été suivie grâce à une entrée placée devant la ruchette (fig 1). Les observations ont été faites tous les jours durant les 2 semaines qui ont suivi l'émergence. Dans tous les cas, le traitement à l'hormone juvénile a provoqué une activité de vol significativement plus précoce des jeunes mâles (tableau I). Les mâles traités à cette hormone ont entrepris des vols en moyenne 2 à 3 jours plus tôt. Ce n'est qu'au bout de 9 jours que l'activité de vol des mâles non traités a atteint la même intensité (fig 2). Cette action de l'hormone juvénile est une preuve que l'activité de vol des abeilles dépend de la teneur en hormone juvénile de l'hémolymphe. Notre interprétation concorde avec les résultats récemment publiés (Tozetto et al, 1995) qui montrent que la synthèse de l'hormone juvénile par les corpora allata est réactivée chez les mâles adultes. Il existe, dans la littérature, des données semblables concernant le passage chez les ouvrières de l'activité d'intérieur à l'activité extérieure de butinage. Les résultats des expériences décrites ici confirment le rôle général de l'hormone juvénile comme stimulant du vol chez l'abeille. On attend encore une telle preuve pour le vol nuptial des reines.

***Apis mellifera carnica* / activité de vol du mâle / hormone juvénile / stimulus / vol précoce**

Zusammenfassung — Juvenilhormon-Behandlung fördert Flugaktivität bei Drohnen (*Apis mellifera carnica*). Um die Hypothese zu prüfen, daß Juvenilhormon bei Bienen generell die Flugaktivität stimuliert, wurden Versuche durchgeführt, bei denen Drohnen 2 - 5 Tage nach dem Schlüpfen einmal topikal mit 5 bzw. 10 µg Juvenilhormon III (JH) behandelt wurden. Die altersmarkierten Drohnen wurden in kleinen Völkern in Kirchhainer Kästchen gehalten. Mittels eines Vorsatzes (Vestibulum) wurde kontrolliert, ob ein Drohn einen Flugversuch unternehmen wollte (Abb 1). Bis 2 Wochen nach dem Schlüpfen wurde dies täglich kontrolliert. Die JH-Behandlung bewirkte in allen Fällen eine signifikant vorzeitige Flugaktivität der jungen Drohnen (Tabelle 1). Die JH-behandelten Drohnen unternahmen um durchschnittlich 2 - 3 Tage verfrühte Flugversuche. Erst vom 9. Lebenstag an waren die Flugaktivitäten der unbe-

handelten Kontrolldrohnen gleich intensiv (Abb 2). Im Zusammenhang mit unseren kürzlich veröffentlichten Befunden, daß in imaginalen Drohnen die JH-Synthese der Corpora allata reaktiviert wird, interpretieren wir diese Wirkung von JH als Beleg für eine generelle Abhängigkeit der Flugaktivität bei Bienen von einem hohen JH-Titer in der Hämolymphe. Entsprechende Analysedaten und Versuchsergebnisse liegen in der Literatur für den Übergang von der Stockbienen- zur Flugbienen-Phase bei Arbeiterinnen vor. Die Ergebnisse unserer hier beschriebenen Experimente bestätigen eine allgemeine Wirkung von JH als Flug-Stimulus bei Bienen. Für den Hochzeitsflug der Bienenköniginnen steht ein solcher Nachweis noch aus.

***Apis mellifera carnica* / Flugaktivität der Drohnen / Juvenilhormon / Flug-Stimulus / vorzeitige Flugversuche**

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